

# Respiratory syncytial virus infection influences tight junction integrity

J. I. Kast,<sup>\*,†</sup> A. J. McFarlane,<sup>‡</sup>

A. Głobińska ,<sup>\*,§</sup>

M. Sokolowska,<sup>\*,†</sup> P. Wawrzyniak,<sup>\*,†</sup>

M. Sanak,<sup>§</sup> J. Schwarze,<sup>‡,\*\*</sup>

C. A. Akdis<sup>\*,†</sup> and K. Wanke<sup>\*,†</sup>

<sup>\*</sup>Swiss Institute of Allergy and Asthma Research, University of Zurich, <sup>†</sup>CK-CARE, Christine Kühne Center of Allergy Research and Education, Davos, Switzerland, <sup>‡</sup>Medical Research Council Centre for Inflammation Research, University of Edinburgh, Edinburgh, UK, <sup>§</sup>Department of Immunology, Rheumatology and Allergy, Medical University of Lodz, Lodz, Poland, <sup>¶</sup>Department of Medicine, Jagiellonian University Medical College, Cracow, Poland, and <sup>\*\*</sup>Child Life and Health, University of Edinburgh, Edinburgh, UK

Accepted for publication 15 August 2017

Correspondence: K. Wanke, Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse 22, 7270 Davos Platz, Switzerland.  
E-mail: wanke.kerstin@gmail.com

## Summary

Respiratory syncytial virus (RSV) is an important risk factor of asthma development and is responsible for severe respiratory tract infections. However, the influence of RSV infection on barrier function of bronchial epithelial cells *in vitro* and *in vivo* is still unclear. The aim of this study was to analyse the role of RSV in tight junction (TJ) regulation and to compare epithelial integrity between asthmatic and healthy individuals upon RSV infection. Healthy and asthmatic human bronchial epithelial cells (HBECs) were differentiated at air–liquid interface (ALI) and infected with RSV and ultraviolet (UV)-irradiated RSV. TJ expression and their integrity were analysed by quantitative polymerase chain reaction (qPCR), transepithelial resistance (TER) and paracellular flux. To determine the effect *in vivo*, BALB/c mice were infected intranasally with RSV or UV-irradiated RSV A2. Bronchoalveolar lavage and TJ integrity were analysed on days 1, 2, 4 and 6 post-infection by qPCR, bioplex and confocal microscopy. RSV increased barrier integrity in ALI cultures of HBEC from healthy subjects, but no effect was found in HBECs from asthmatics. This was not associated with an increase in TJ mRNA expression. *In vivo*, RSV induced lung inflammation in mice and down-regulated claudin-1 and occludin mRNA expression in whole lungs. Surprisingly, RSV infection was not observed in bronchial epithelial cells, but was found in the lung parenchyma. Decreased expression of occludin upon RSV infection was visible in mouse bronchial epithelial cells in confocal microscopy. However, there was no regulation of claudin-1 and claudin-7 at protein level.

**Keywords:** airway epithelial cells, asthma, respiratory syncytial virus, tight junctions

## Introduction

Human respiratory syncytial virus (RSV) is a negative-sense, single-stranded virus which belongs to the *Paramyxoviridae* family. RSV not only infects epithelial cells in the upper airways of adults, but is also the leading cause of lower respiratory tract infections in infants below the age of 2 years [1]. Infections in children result in more severe lower respiratory tract inflammation, leading in some cases to severe bronchiolitis and pneumonia and hospitalization [2–4]. In addition, RSV infection in childhood has been linked to recurrent wheeze, induction of childhood asthma and sensitization to allergens [5–9]. However, it is unclear how RSV and other respiratory viruses propagate *in vivo* and how RSV is linked to the development of asthma and

allergies. In the case of rhinovirus infection, asthmatics display impaired interferon (IFN) production in response to infection [10,11] but, conversely, no IFN deficiency was observed in well-controlled asthma [12]. In addition, a recent study suggested that there is no difference in IFN response to RSV between asthmatic and non-asthmatic individuals [13]. However, a study on hospitalized infants infected with RSV demonstrated diminished IFN- $\gamma$  production at the time of bronchiolitis. Reduced IFN- $\gamma$  production was related to development of asthma after bronchiolitis in infants [14].

Infection with RSV induces secretion of cytokines and chemokines from epithelial cells and resident leucocytes, which leads in turn to recruitment of circulating leucocytes

to the infected lungs [15]. It has been demonstrated that RSV stimulates release of chemokines, i.e. regulated upon activation, normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein 1 (MIP-1 $\alpha$ ), MIP-1 $\beta$ , monocyte chemoattractant protein-1 (MCP-1) and keratinocyte-derived chemokine (KC), as well as cytokines such as granulocyte-colony stimulating factor (G-CSF), interleukin (IL)-6 and IL-1 $\beta$  [16–18].

Tight junctions (TJ) form an essential part of the barrier between the mucosa or skin and the environment by connecting adjacent epithelial cells. A continuous band of TJ surrounds every epithelial cell and builds the most apical, extracellular cell–cell adhesion complex. TJ consist of large, transmembrane protein complexes with members of the claudin family, the MAL and related proteins for vesicle trafficking and membrane link (MARVEL) family and the junctional adhesion molecule (JAM) family, which connect adjacent cells [19,20]. Cell types from different human tissues express a unique pattern of TJ proteins according to their specialization [21]. TJ are important in barrier formation. They prevent particles and pathogens from penetrating the tissue, control extracellular fluid, paracellular flux of molecules and provide the apico-basolateral axis of differentiation [22]. Epithelial barrier defects due to disruptions in TJ have been reported in several allergic and inflammatory diseases, such as atopic dermatitis, asthma and chronic rhinosinusitis, and a role of TJ in smooth muscle cells in asthma has been described [23–28]. The connection between RSV infections, barrier function and the impact of the virus on asthma development has not been studied thoroughly. So far, RSV-induced disruption of barrier function in a bronchial epithelial cell line and contradictory results of induction of TJ molecules in human nasal epithelial cells have been reported [29,30]. Although there is some evidence of epithelial barrier dysfunction in asthma [31,32], it is still not clear whether RSV infection may have an influence on TJ in asthma.

Therefore, the aim of this study was to identify the role of RSV in TJ regulation and function of epithelial integrity *in vitro* in asthmatic and healthy bronchial epithelial cell cultures. We also sought to explore the course of RSV infection *in vivo* using a mouse model to mimic the situation in which RSV infection precedes asthma development and analyse here the inflammatory response and the regulation of TJ.

## Material and methods

### RSV propagation

Human epithelial type 2 (HEp2) cells,  $5 \times 10^6$ /ml, were incubated overnight at 37°C, 5% CO<sub>2</sub> in complete Dulbecco's modified Eagle's medium (DMEM)/F12 medium. After 24 h, the cells were washed with serum-free DMEM/F12 medium. The cultures were infected with human RSV

[type A, strain A2 (ATCC, Teddington, UK)] at a virus concentration of 0.1 plaque-forming units (pfu)/cell and cultured in serum-free DMEM/F12 at 37°C, 5% CO<sub>2</sub> for 48 h. After this time, the cytopathic effect was assessed and the cells were harvested using a cell scraper. Cultures were centrifuged and the resultant supernatant was snap-frozen as viral stock in liquid nitrogen. Viral stocks were kept at –80°C until use. Control flasks of uninfected HEp2 cells were treated in the same way as control supernatants.

### Isolation of primary bronchial epithelial cells and ALI cultures

Primary human bronchial epithelial cells (HBECs) were obtained from asthmatic patients undergoing bronchoscopy and bronchial brushings after written informed consent by each patient. Healthy HBECs were bought from Lonza (Basel, Switzerland). Cells were removed from brushes, seeded into collagen and fibronectin-coated 25 cm<sup>2</sup> T flasks and cultured in basal epithelial growth medium (BEGM; Lonza) at 37°C, 5% CO<sub>2</sub>. Cells ( $1.5 \times 10^5$ ) of passages 3 or 4 were seeded into collagen-precoated (Sigma, St Louis, MO, USA) 24-well, polyester, 6.5 mm Transwell plates (Corning, Inc., New York, NY, USA) with 0.4  $\mu$ m pore size in air–liquid interface (ALI) medium [DMEM (GIBCO, Carlsbad, CA, USA) 1 : 1 mixed with BEGM without triiodothyronine and freshly substituted with transretinoic acid (Sigma)]. Apical medium was removed after 4 days. Because our previous experiments [33] have demonstrated lower transepithelial resistance (TER) values in HBECs from asthmatic donors compared to control subjects, cells were used for the experiment when ALI cultures from healthy donors reached at least  $330 \Omega \times \text{cm}^2$  and asthmatic HBECs at least  $200 \Omega \times \text{cm}^2$  in TER measurement with electrodes and a Millicell ERS Volt-Ohm Meter (Millipore, Billerica, MA, USA). The six healthy and six asthmatic cultures were infected apically with RSV [multiplicity of infection (MOI) = 1.5 or 0.15] or ultraviolet (UV)-irradiated RSV (UV-RSV) (MOI = 1.5) or incubated with control medium (u.s.). After 2 h the cultures were washed three times and continued to be cultured at ALI.

### RSV mouse model

Wild-type BALB/c mice, 8–12 weeks old, were maintained under specific pathogen-free conditions. Mice were housed at the Medical Research Council Centre for Inflammation Research, University of Edinburgh, UK in individually ventilated cages for the duration of the study, and all experimental procedures were carried out in accordance with Home Office regulations. Mice were infected intranasally with  $7.4 \times 10^5$  pfu RSV A2 or UV-irradiated RSV, or left naive. Mice were euthanized at days 1, 2, 4 and 6 after infection (Supporting information, Fig. S1a). Group size was eight and the experiment was repeated three times.

### Bronchoalveolar lavage (BAL) protein concentration and bioplex analysis

BAL was obtained by injecting 1 ml of phosphate-buffered saline (PBS) containing protease inhibitor (Roche, Basel, Switzerland) into the mouse lung and retrieving usually 650–900 µl of injected fluid. The BAL supernatant was collected and total protein concentration was measured by a standard Bradford assay (BioRad, Hercules, CA, USA). Bio-plex analysis of the BAL was performed with Bio-Plex Pro™ Mouse Cytokine 23-plex Assay with the Bio-Plex 200 System (BioRad).

### mRNA isolation and quantitative reverse transcription–polymerase chain reaction (quantitative realtime PCR)

Lung samples were stored directly in RNeasy lysis buffer (Qiagen, Valencia, CA, USA) and frozen for later analyses. Lungs were then shredded with ceramic beads in RLT buffer (Precellys®24 tissue homogenizer; Bertin Technologies, Montigny-le Bretonneux, France). Cells from ALI cultures at day 6 after infection were lysed directly in RLT buffer, and RNA was isolated subsequently with the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. cDNA was prepared with reverse transcription reagents containing random hexamer primers (Fermentas, Waltham, MA, USA). For relative quantification, cDNA was amplified in the presence of SYBR Green and detected by an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Primer sequences for human and mouse primers can be found in Supporting information, Table S1. For human samples elongation factor 1 $\alpha$  (EF1 $\alpha$ ) and for mouse samples glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or EF1 $\alpha$  were used as internal control. Relative expression of mRNA was calculated using the comparative  $\Delta\Delta$ CT-method [34].

### Lung histology and immunofluorescence staining of ALI and lung sections

For confocal microscopy, lung samples were frozen in Tissue Tek (Sakura Finetek, Alphen aan den Rijn, the Netherlands) and cut into 30-µm thick sections with a cryomicrotome (Microm; Carl Zeiss, Oberkochen, Germany). Lung sections were fixed with 4% paraformaldehyde, blocked and permeabilized with PBS containing 10% donkey serum (Sigma-Aldrich, St Louis, MO USA), 1% bovine serum albumin (BSA) (Sigma-Aldrich), 0.1% Triton-X 100 (Acros Organics, Geel, Belgium) and 0.02% sodium dodecyl sulphate (SDS) (Sigma-Aldrich). Samples were stained with Alexa488-labelled mouse anti-occludin, polyclonal rabbit anti-claudin-1, polyclonal rabbit anti-claudin-7 and polyclonal goat anti-RSV (AbD Serotec, Hercules, CA, USA) antibodies and detected with secondary antibodies labelled with Alexa-488, 546 or 633 (Invitrogen,

Carlsbad, CA, USA). Stainings were mounted in ProLong-Gold with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen) and analysed with a Leica TCS SPE confocal microscope (Leica Microsystems, Wetzlar, Germany).

### Statistical analysis

Non-parametric data were analysed using the Mann–Whitney *U*-test, while paired data sets were analysed using the Wilcoxon matched-pair test. Spearman's correlation test was used between RSV load and chemokines, cytokines and TJ. All statistical analysis was conducted using the GraphPad Prism version 5c software. A *P*-value < 0.05 was considered statistically significant.

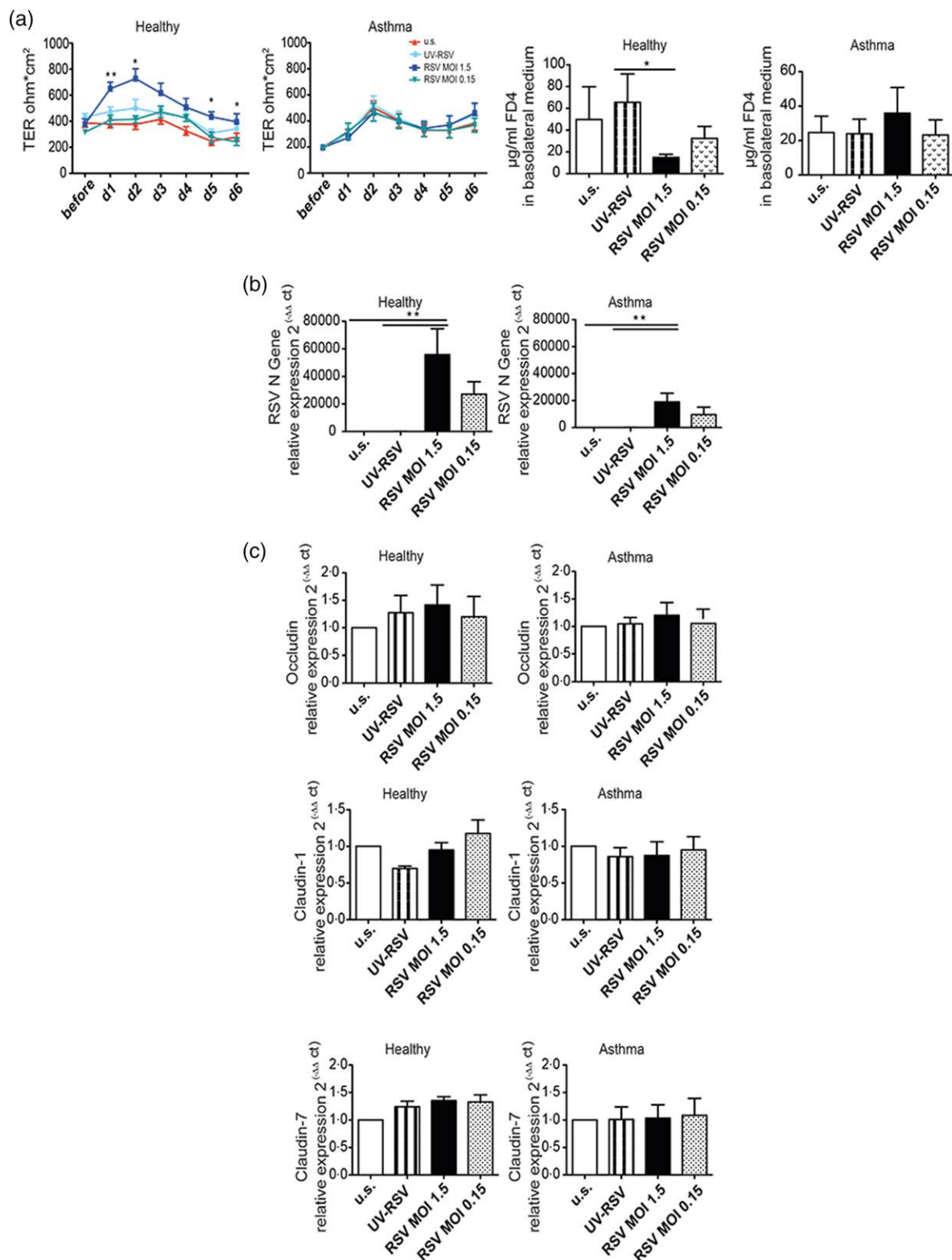
## Results

### Increased barrier response to RSV in healthy, but not in asthmatic bronchial epithelial cells

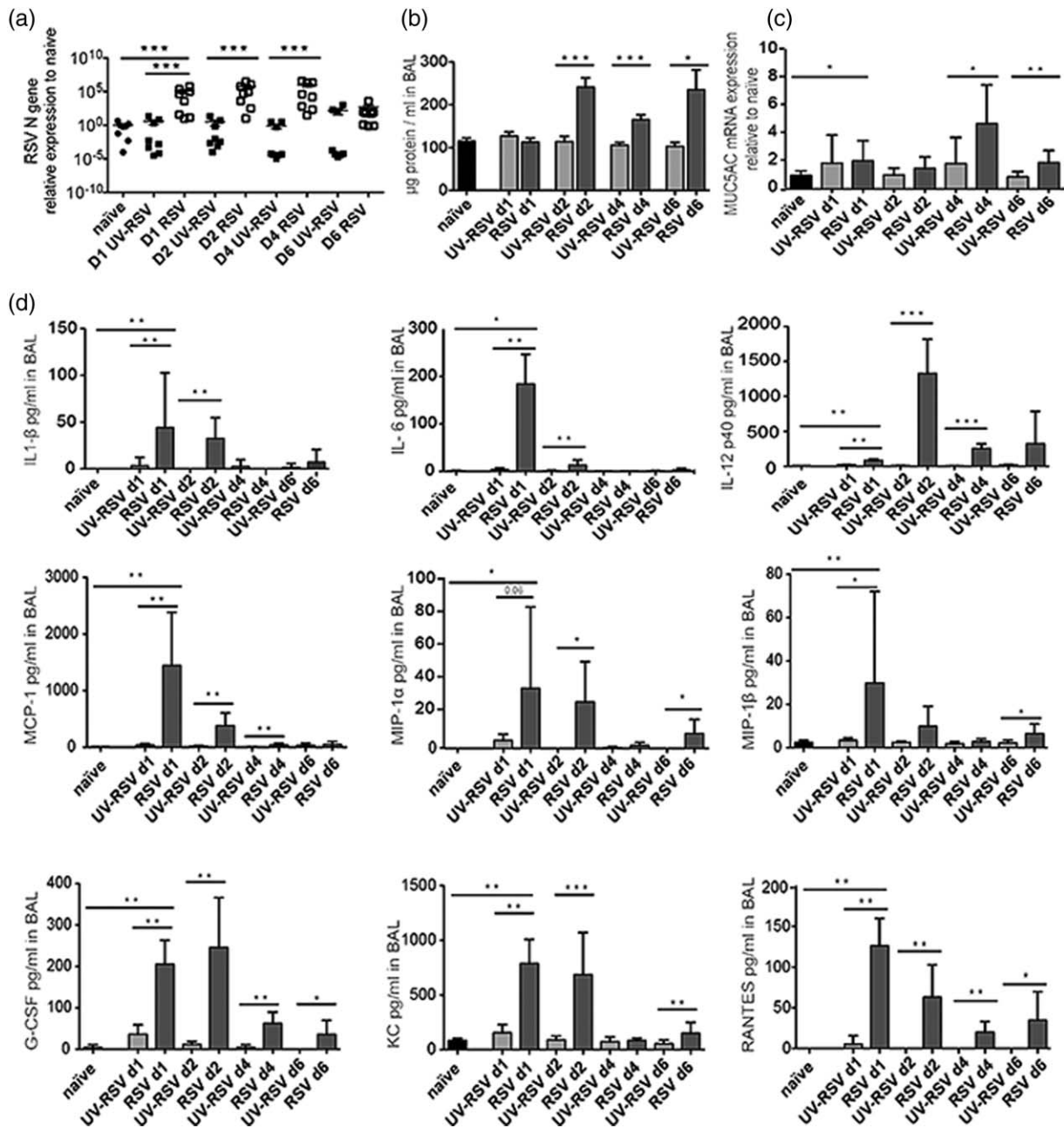
To determine the direct role of RSV on TJ of healthy and asthmatic HBECs, cells were cultured under ALI conditions and infected with RSV MOI of 0.15 or 1.5. Controls were treated either with medium or UV-RSV. As reported previously, we also noted that the baseline epithelial integrity is compromised in asthmatics compared to controls [33]. We demonstrated here that RSV increased transepithelial resistance (TER) and decreased paracellular flux in healthy, but not in asthmatic subjects (Fig. 1a). As a control, we confirmed RSV infection of healthy and asthmatic HBECs (Fig. 1b). On day 6 post-infection, HBEC cultures were harvested and analysed. Interestingly, virus load in asthmatic HBECs seemed to be lower than that observed in healthy individuals, but this difference was not statistically significant. The expression of the TJ genes – occludin, claudin-1 and claudin-7 – were not regulated significantly on mRNA levels in both groups (Fig. 1c). Thus, our findings suggest that healthy HBECs' response to RSV leads to an increase in epithelial barrier integrity reflected by decreased paracellular flux and enhanced transepithelial resistance, which does not occur in asthmatic patients. However, this effect was not due to a change in the expression of TJ molecules mRNA in HBECs.

### RSV infected mouse lungs and induced inflammation

As in the clinic RSV infections in infants usually precede asthma development, we aimed to investigate the effect on lung epithelial barrier function *in vivo*. For this reason, mice were inoculated intranasally with RSV or UV-RSV. Mouse lungs and BAL were harvested on days 1, 2, 4 and 6 post-infection (Supporting information, Fig. S1a). Confirming the RSV infection in the lungs, we found high viral gene expression in RSV-infected mice, whereas there was no infection in control naive mice or in the UV-RSV-receiving control group at day 1 after infection, persisting



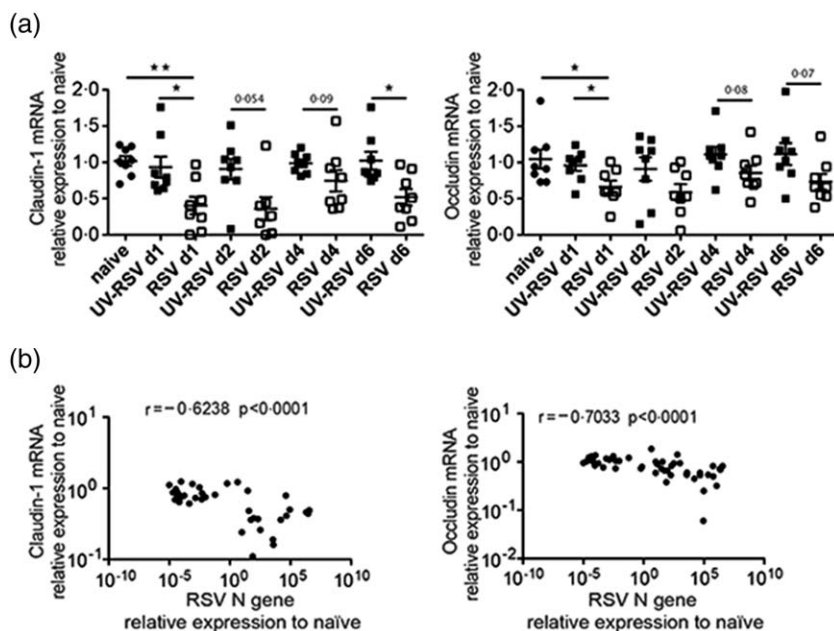
**Fig. 1.** Infection of human healthy and asthmatic normal human bronchial epithelial cells (NHBE) with respiratory syncytial virus (RSV) and its effect on tight junction (TJ). (a) Transepithelial resistance (TER) analysis and paracellular flux analysis of healthy ( $n = 6$ ) and asthmatic ( $n = 6$ ) bronchial epithelial cell cultures infected with ultraviolet (UV)-RSV, RSV multiplicity of infection (MOI) = 0.15, RSV MOI = 1.5 and medium control (u.s.). (b) Quantitative polymerase chain reaction (qPCR) analysis of RSV N gene to determine the viral load of these cultures at day 6 after infection. (c) qPCR analysis of occludin, claudin-1 and claudin-7 of these cultures at day 6 after infection. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**Fig. 2.** Respiratory syncytial virus (RSV) infection leads to inflammation in the lungs. (a) Quantitative polymerase chain reaction (qPCR) analysis of RSV N gene in mouse lungs after the infection with RSV to determine the viral load. (b) Bradford assay of bronchoalveolar (BAL) to show protein influx into the lung lumen and (c) mucin 5AC (MUC5AC) mRNA expression in the lung in naive and post-infection with RSV. (d) Level of interleukin (IL)-1 $\beta$ , IL-6, IL-12 p40, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory proteins (MIP)-1 $\alpha$ , MIP-1 $\beta$ , granulocyte-colony-stimulating factor (G-CSF), keratinocyte-derived chemokine (KC) and regulated on activation normal T cell expressed and secreted (RANTES) in BAL;  $n = 8$ , experiments were repeated three times.

up to day 4 (Fig. 2a). To investigate the influence of viral infection on barrier integrity and to gain an insight into the inflammatory process in the lung, we performed a standard *in-vivo* BAL procedure and analysis. An increased protein influx from the lung parenchyma to the BAL on days 2, 4 and 6 indicated a reduction of the epithelial barrier integrity (Fig. 2b). Furthermore, we found an increased

expression of mucin 5AC (MUC5AC) mRNA on days 1, 4 and 6 upon RSV infection, which suggested an induction of innate immune response in the epithelium (Fig. 2c). In parallel, MUC5AC mRNA expression and overall increase in BAL protein content correlated with the viral load (Supporting information, Fig. S1b). Among the BAL proteins there were proinflammatory cytokines and



**Fig. 3.** TJ gene expression is regulated by respiratory syncytial virus (RSV) in mouse lungs. (a) Quantitative polymerase chain reaction (qPCR) analysis of claudin-1 and occludin in the lung post infection with RSV compared to naïve and (b) their correlation with the RSV load;  $n = 8$ , experiments were repeated three times.

chemokines, such as IL-1 $\beta$ , IL-6, IL-12 p40, MCP-1, MIP-1 $\alpha$  and MIP-1 $\beta$ , G-CSF, KC and RANTES, most of which increased significantly upon RSV infection at days 1, 2, 4 and 6 (Fig. 2d). Released chemokines and cytokines correlated positively with RSV load in the lung (Supporting information, Fig. S1c). In conclusion, RSV infection *in vivo* induced active lung inflammation and reduced airway epithelial barrier function.

### RSV infection down-regulated TJ in mouse lungs

To characterize further the mechanisms of barrier dysfunction upon RSV infection *in vivo*, detailed qPCR analysis of TJ in RSV and UV-RSV-infected mice was performed. In mouse lungs, a distinct set of TJ was found. All claudin family members, except claudin-2 and claudin-6, the MARVEL family members – occludin, tricellulin and marvelD3 splice variant 2 (MarvelD3.2), as well as the junctional adhesion molecules -JAM-A, -B and -C and plaque protein zonula occludens (ZO) 1 genes were expressed (Supporting information, Fig. S2a). Claudin-1 was down-regulated significantly on days 1 and 6 post-infection and there was a trend towards the decrease on days 2 and 4. Occludin was down-regulated significantly only on day 1 and showed a tendency to be down-regulated on days 4 and 6 (Fig. 3a). In addition, claudin-1 and occludin correlated negatively with RSV load in the lungs of infected mice (Fig. 3b). In contrast, claudin-7 was up-regulated in RSV-infected compared to naïve mice on day 1, but did not correlate with the RSV load (Supporting information, Fig. S2b). Other TJ, such as claudin-10b and tricellulin, were down-regulated significantly compared to naïve at day 1 after infection (Supporting information, Fig. S2c). In conclusion, RSV infection led to the down-regulation of claudin-

1 and occludin mRNA in mouse lungs, which correlated with barrier dysfunction and viral load.

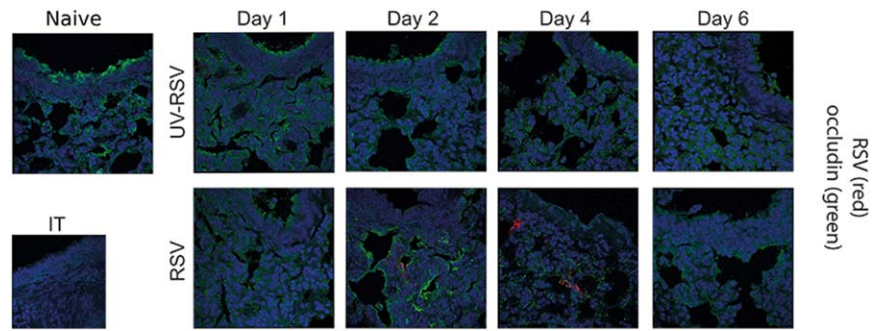
### RSV was found only in the lung parenchyma and did not regulate TJ directly in mouse bronchial epithelial cells

To determine the regulation of TJ and location of RSV infection in mouse lungs, immunofluorescence staining of TJ and RSV envelope proteins was performed. Interestingly, we demonstrated that already at day 1 post-infection, RSV was located in the lung parenchyma, but was not found in the bronchial epithelial cells (Fig. 4, Supporting information, Fig. S3). Moreover, we found that occludin expression in the bronchial epithelium was lower in the RSV-infected mouse compared to UV-RSV at days 2 and 4 (Fig. 4) and corresponded to occludin mRNA expression level, correlation with viral load and increased protein leak to the BAL at day 2. However, there was no visible regulation of the protein expression of claudin-1 (Supporting information, Fig. S3a) or claudin-7 (Supporting information, Fig. S3b), suggesting that the stability of these proteins might be greater than occludin and that they are additionally highly regulated at the post-transcriptional level. To conclude, RSV infection correlated with the decreased occludin expression in the bronchial epithelium, while the expression of other TJ proteins was influenced at the transcriptional level in the whole lungs.

### Discussion

The aim of this study was to investigate the role of RSV infection in TJ regulation. We first sought to investigate the effect of RSV on TJ in asthmatic HBECs and healthy

**Fig. 4.** Respiratory syncytial virus (RSV) is located in the mouse lung parenchyma. Representative confocal staining of occludin (green) and RSV (red) post infection with RSV and naive control mouse lung. 4',6-diamidino-2-phenylindole (DAPI) is stained in blue. Experiments were repeated three times. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



controls, and secondly, to study RSV effects on TJ integrity in mouse model of infection, which usually precedes asthma development.

We found that RSV infection of primary HBECs led to an increase in barrier function in healthy, but not in asthmatic subjects. This was reflected by the change in barrier function parameters (TER, flux), but not by TJ gene expression level, suggesting very early innate response to RSV infection only in healthy epithelium. It is possible that T helper type 2 (Th2) inflammation ongoing in allergic asthmatic patients alters epithelial cell function and precludes barrier tightening in response to RSV. This hypothesis is supported by the fact that normal bronchial epithelial cells stimulated with IL-4 and IL-13 demonstrate significant decrease in barrier integrity similar to that observed in HBECs from asthmatic patients [33]. Additionally, the Th2 environment may interfere with the viral replication. Virus load at day 6 post-infection in asthmatic HBECs was lower compared to healthy individuals HBECs. Similarly, a significantly diminished proliferation of parainfluenza virus (another *Paramyxoviridae* family member) has been demonstrated in nasal epithelial cells from allergic individuals [35]. These results suggest that already compromised barrier function in asthmatics is unable to react efficiently to RSV infection and provides natural defence mechanisms; however, a decrease in barrier function does not result in the increase of virus load in asthmatic HBECs.

Thus, we observed here an additional dysfunction of asthmatic epithelium, which extends other observations of impaired innate mechanisms in response to viral infections [36,37]. These data are in line with previous observations that RSV infection of human nasal epithelial cells results in enhanced expression of claudin-4 and occludin, inducing cell polarity, which may facilitate virus budding [30,38].

The next aim of our study was to examine the influence of RSV on barrier function *in vivo* in a mouse model of RSV infection. We found that RSV was present only in the lung parenchyma, whereas the murine bronchial epithelial cells did not appear to be infected. We hypothesize that RSV might influence TJ expression in lung parenchyma cells either directly upon replication or indirectly via activation of inflammatory mediators. A similar RSV infection pattern has been found in children with severe RSV

infection [2,39]. This effect may be associated with the immature immune response and smaller physical dimensions of children's airways, which may be a potential explanation of the viral location in the infant's lungs [40]. Therefore, our results suggest that the mouse model of RSV infection may mimic the course of infection in infants, which is related to disruption of the distal airway epithelium.

Conversely, an RSV-induced barrier response in primary bronchial epithelial cells from adults might not be reflected fully in the mouse model of infection. It has been demonstrated that BALB/c mice used in our study are semi-permissive to RSV infection and develop only limited inflammation [41]. In view of differences in the immune response and physical differences of lung structure between adults and infants, localization of the infection in adults is not limited to the lung parenchyma, but rather located in the upper airway epithelium. Therefore, being aware of the fact that the mouse model of RSV infection may not reflect the range of pathologies observed in humans, we do not compare directly the results obtained *in vitro* with the *in vivo* mouse model. It is possible that using another rodent model of RSV infection, i.e. cotton rats, we would be able to detect virus in epithelial cells of the lungs [42,43]. Conversely, *in-vitro* experiments with one type of cells might reflect only the early response of epithelium to the replication of virus and probably do not reflect fully other events during ongoing RSV-induced inflammation. Thus, we believe that the results obtained from both experimental models complement each other and provide different information: (i) in contrast to healthy controls, adult asthmatic epithelium is not able to provide resistance to RSV infection by tightening the already compromised TJs barrier; and (ii) *in-vivo* RSV-infection and the following RSV-induced inflammation decrease whole lung barrier function, which might possibly contribute to the further development of asthma.

In summary, our data demonstrated that RSV infection increased barrier integrity in healthy subjects, while the mechanism was impaired in asthmatic bronchial epithelial cells. We have also demonstrated that the *in-vivo* mouse model RSV is located in lung parenchyma and RSV infection dysregulated TJ expression and function in the whole

lung. However, further studies are needed to elucidate the link between RSV infection and barrier function.

## Acknowledgements

This work was supported by Swiss National Science Foundation (SNF) grant number 310030-156823.

## Disclosure

The authors declare that there are no conflicts of interest.

## References

- Johnson JE, Gonzales RA, Olson SJ, Wright PF, Graham BS. The histopathology of fatal untreated human respiratory syncytial virus infection. *Mod Pathol* 2007; **20**:108–19.
- Pickles RJ, DeVincenzo J. RSV and its propensity for causing bronchiolitis. *J Pathol* 2014; **235**:266–76.
- Lambert L, Sagfors AM, Openshaw PJ, Culley FJ. Immunity to RSV in early-life. *Front Immunol* 2014; **5**:466.
- Tovey ER, Stelzer-Braid S, Toelle BG *et al.* Rhinoviruses significantly affect day-to-day respiratory symptoms of children with asthma. *J Allergy Clin Immunol* 2015; **135**:663–9.e12.
- Tourdot S, Mathie S, Hussell T *et al.* Respiratory syncytial virus infection provokes airway remodelling in allergen-exposed mice in absence of prior allergen sensitization. *Clin Exp Allergy* 2008; **38**:1016–24.
- Schwarze J. Lung dendritic cells in respiratory syncytial virus bronchiolitis. *Pediatr Infect Dis J* 2008; **27**:S89–91.
- Schwarze J, Makela M, Cieslewicz G *et al.* Transfer of the enhancing effect of respiratory syncytial virus infection on subsequent allergic airway sensitization by T lymphocytes. *J Immunol* 1999; **163**:5729–34.
- Schwarze J, Hamelmann E, Bradley KL, Takeda K, Gelfand EW. Respiratory syncytial virus infection results in airway hyperresponsiveness and enhanced airway sensitization to allergen. *J Clin Invest* 1997; **100**:226–33.
- James KM, Gebretsadik T, Escobar GJ *et al.* Risk of childhood asthma following infant bronchiolitis during the respiratory syncytial virus season. *J Allergy Clin Immunol* 2013; **132**:227–9.
- Wark PA, Johnston SL, Buchieri F *et al.* Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005; **201**:937–47.
- Gielen V, Sykes A, Zhu J *et al.* Increased nuclear suppressor of cytokine signaling 1 in asthmatic bronchial epithelium suppresses rhinovirus induction of innate interferons. *J Allergy Clin Immunol* 2015; **136**:177–88.e11.
- Sykes A, Macintyre J, Edwards MR *et al.* Rhinovirus-induced interferon production is not deficient in well controlled asthma. *Thorax* 2014; **69**:240–6.
- Patel DA, You Y, Huang G *et al.* Interferon response and respiratory virus control are preserved in bronchial epithelial cells in asthma. *J Allergy Clin Immunol* 2014; **134**:1402–12.e7.
- Renzi PM, Turgeon JP, Marcotte JE *et al.* Reduced interferon-gamma production in infants with bronchiolitis and asthma. *Am J Respir Crit Care Med* 1999; **159**:1417–22.
- Tripp RA, Oshansky C, Alvarez R. Cytokines and respiratory syncytial virus infection. *Proc Am Thorac Soc* 2005; **2**:147–9.
- Miller AL, Bowlin TL, Lukacs NW. Respiratory syncytial virus-induced chemokine production: linking viral replication to chemokine production *in vitro* and *in vivo*. *J Infect Dis* 2004; **189**:1419–30.
- Murai H, Terada A, Mizuno M *et al.* IL-10 and RANTES are elevated in nasopharyngeal secretions of children with respiratory syncytial virus infection. *Allergol Int* 2007; **56**:157–63.
- Oshansky CM, Barber JP, Crabtree J, Tripp RA. Respiratory syncytial virus F and G proteins induce interleukin 1alpha, CC, and CXC chemokine responses by normal human bronchoepithelial cells. *J Infect Dis* 2010; **201**:1201–7.
- Chiba H, Osanai M, Murata M, Kojima T, Sawada N. Transmembrane proteins of tight junctions. *Biochim Biophys Acta* 2008; **1778**:588–600.
- Furuse M. Molecular basis of the core structure of tight junctions. *Cold Spring Harb Perspect Biol* 2010; **2**:a002907.
- Kast JI, Wanke K, Soyka MB *et al.* The broad spectrum of interepithelial junctions in skin and lung. *J Allergy Clin Immunol* 2012; **130**:544–7.e4.
- Schulzke J-D, Günzel D, John LJ, Fromm M. Perspectives on tight junction research. *Ann N Y Acad Sci* 2012; **1257**:1–19.
- De Benedetto A, Rafaels NM, McGirt LY *et al.* Tight junction defects in patients with atopic dermatitis. *J Allergy Clin Immunol* 2011; **127**:773–86.e7.
- de Boer WI, Sharma HS, Baelemans SM, Hoogsteden HC, Lambrecht BN, Braunstahl GJ. Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. *Can J Physiol Pharmacol* 2008; **86**:105–12.
- Fujita H, Chalubinski M, Rhyner C *et al.* Claudin-1 expression in airway smooth muscle exacerbates airway remodeling in asthmatic subjects. *J Allergy Clin Immunol* 2011; **127**:1612–21.e8.
- Holgate ST. Epithelium dysfunction in asthma. *J Allergy Clin Immunol* 2007; **120**:1233–44; quiz: 45–6.
- Soyka MB, Wawrzyniak P, Eiwegger T *et al.* Defective epithelial barrier in chronic rhinosinusitis: the regulation of tight junctions by IFN- $\gamma$  and IL-4. *J Allergy Clin Immunol* 2012; **130**:1087–96.e10.
- Xiao C, Puddicombe SM, Field S *et al.* Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011; **128**:549–56.e12.
- Rezaee F, DeSando SA, Ivanov AI *et al.* Sustained protein kinase D activation mediates respiratory syncytial virus-induced airway barrier disruption. *J Virol* 2013; **87**:11088–95.
- Masaki T, Kojima T, Okabayashi T *et al.* A nuclear factor- $\kappa$ B signaling pathway via protein kinase C  $\delta$  regulates replication of respiratory syncytial virus in polarized normal human nasal epithelial cells. *Mol Biol Cell* 2011; **22**:2144–56.
- Sweerus K, Lachowicz-Scroggins M, Gordon E *et al.* Claudin-18 deficiency is associated with airway epithelial barrier dysfunction and asthma. *J Allergy Clin Immunol* 2016; **139**:72–81.e1.
- Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. *J Allergy Clin Immunol* 2014; **134**:509–20.
- Wawrzyniak P, Wawrzyniak M, Wanke K *et al.* Regulation of bronchial epithelial barrier integrity by type 2 cytokines and histone deacetylases in asthmatic patients. *J Allergy Clin Immunol* 2016; **139**:93–103.
- Kim DW. Real time quantitative PCR. *Exp Mol Med* 2001; **33**:101–9.

- 35 Globinska A, Pawelczyk M, Piechota-Polanczyk A *et al*. Impaired virus replication and decreased innate immune responses to viral infections in nasal epithelial cells from patients with allergic rhinitis. *Clin Exp Immunol* 2017; **187**: 100–12.
- 36 Sokolowska M, Chen LY, Liu Y *et al*. Dysregulation of lipidomic profile and antiviral immunity in response to hyaluronan in patients with severe asthma. *J Allergy Clin Immunol* 2016; **139**: 1379–83.
- 37 Sokolowska M, Stefanska J, Wodz-Naskiewicz K, Cieslak M, Pawliczak R. Cytosolic phospholipase A2 group IVA is overexpressed in patients with persistent asthma and regulated by the promoter microsatellites. *J Allergy Clin Immunol* 2010; **125**: 1393–5.
- 38 Tsutsumi H, Kojima T, Hirakawa S *et al*. Respiratory syncytial virus infection and the tight junctions of nasal epithelial cells. *Adv Otorhinolaryngol* 2011; **72**:153–6.
- 39 Fonceca AM, Flanagan BF, Trinick R, Smyth RL, McNamara PS. Primary airway epithelial cultures from children are highly permissive to respiratory syncytial virus infection. *Thorax* 2012; **67**: 42–8.
- 40 Pickles RJ, DeVincenzo JP. Respiratory syncytial virus (RSV) and its propensity for causing bronchiolitis. *J Pathol* 2015; **235**: 266–76.
- 41 Graham BS, Perkins MD, Wright PF, Karzon DT. Primary respiratory syncytial virus infection in mice. *J Med Virol* 1988; **26**: 153–62.
- 42 Lemon K, Nguyen DT, Ludlow M *et al*. Recombinant subgroup B human respiratory syncytial virus expressing enhanced green fluorescent protein efficiently replicates in primary human cells and is virulent in cotton rats. *J Virol* 2015; **89**:2849–56.
- 43 Taylor G. Animal models of respiratory syncytial virus infection. *Vaccine* 2017; **35**:469–80.

## Supporting information

Additional Supporting information may be found in the online version of this article at the publisher's web-site:

**Fig. S1.** (a) Experimental protocol. BALB/c mice were infected intranasally with  $7.4 \times 10^5$  plaque-forming units (PFU) respiratory syncytial virus (RSV) A2 or ultraviolet (UV)-irradiated RSV or left naive, and lung tissue was analysed at 1, 2, 4 and 6 days post-infection with RSV A2,  $n = 8$ . Experiments were repeated three times. (b) Correlations of protein influx and mucin 5AC (MUC5AC) mRNA with the RSV load. (c) Correlation of interleukin (IL)-1 $\beta$ , IL-6, IL-12p40, monocyte chemotactic protein 1 (MCP)-1, macrophage inflammatory proteins (MIP)-1 $\alpha$ , MIP-1 $\beta$ , granulocyte-colony-stimulating factor (G-CSF), keratinocyte-derived chemokine (KC) and regulated on activation normal T cell expressed and secreted (RANTES) with the viral load. All detectable values of the each experiment are included in the graphs.

**Fig. S2.** (a) Quantitative polymerase chain reaction (qPCR) analysis of the mRNA expression of the claudin, Marvel, junctional adhesion molecules (JAM) and zonula occludens (ZO) families in naive mouse lungs. (b) Claudin-7 gene expression and its correlation with the viral load. (c) Claudin-4, claudin-10b and tricellulin gene expression.

**Fig. S3.** Confocal microscopy of (a) claudin-1 (green) and (b) claudin-7 (green) and respiratory syncytial virus (RSV) (red) in naive, ultraviolet (UV)-RSV and RSV-inoculated mice.

**Table S1.** Primer sequences used in this study